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13 ABSTRACT (Maximum 200 words)

The work has been going quite well. We find that the phasic activation of LC cells by target cues in our vigilance task does not occur during epochs of high tonic LC activity, which also corresponds with poor performance (longer latency bar responses and more false alarms). These results lead us now to speculate that the phasic responses of LC neurons to targets may help in discrimination between target and non-target cues (d'), while the elevated tonic activity may increase the overall tendency of the animal to respond behaviorally to any stimulus (B). We also have some preliminary data indicating that activation of LC with pilocarpine decreases attentiveness to the task (as measured by fixation frequency). Finally, we are finding close relationships between LC activity and pupil diameter during the task, revealing a close correspondence with autonomic arousal.

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PRINCIPAL INVESTIGATOR: Gary Aston-Jones, Ph.D.

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OBJECTIVES:

(Previous Statement of Work). Our work supported by AFOSR has initiated the study of cellular mechanisms underlying vigilance and selective behavioral responsivity in primates. We have established a behaving primate preparation for recording discharge of locus coeruleus (LC) neurons in brain during performance of a vigilance task that resembles those used in human psychophysical studies. In the present application we propose to continue and extend these studies. (1) We will record monkey LC unit activity during a vigilance task modified to allow a wide range of stimulus presentation and difficulty. Also, eye position and pupillary diameter will be continuously monitored throughout recordings to ascertain (a) trials during which the animal attends to the task and detects sensory cues (gaze directed at task stimuli) vs. those in which attention is directed elsewhere, and (b) concurrent activity in the autonomic nervous system (reflected in pupillary diameter), a measure of stress response during the task and a possibly important concomitant of central systems in mediating adaptive behavioral responsivity. (2) We will monitor cortical electrical events, termed event-related potentials (ERPs), thought to reflect selective processing of meaningful sensory stimuli, and investigate the role of LC in generating these cortical signals in two ways: (a) simultaneous ERP and LC unit recordings will determine the temporal association between these two events; and (b) local microinjections of selective pharmacologic agents will be used to transiently and specifically inactivate or activate NE-LC neurons while recording ERPs. Such specific manipulations of LC will also allow analysis of vigilance behavior while LC is either inactivated or activated. (3) We will challenge animals' performance by varying task parameters and introducing distractors and environmental stressors that are known to influence vigilance in humans. LC and ERP activity will be monitored, and in other experiments LC will be selectively activated or inactivated, to test the role of this system in mediating adaptive behavioral responsivity under adverse conditions.

The proposed studies will examine in detail both the temporal association (via LC recordings) and functional dependency (via LC activation and inactivation) between the brain noradrenergic LC system, higher-order attentional processing (ERPs), and vigilance performance during normative as well as during stressful conditions. Results of these experiments will open the way to examination of afferents to LC in future studies, to understand circuits and mechanisms involved in determination and processing of the specific stimulus attributes (novel, unexpected, or threatening) that activate LC.

STATUS OF RESEARCH EFFORT:

This section reviews our progress on this project during the previous award. As reviewed below, major progress included (i) improvement of techniques to record LC discharge in behaving monkeys, (ii) further characterization of monkey LC activity during naturalistic behaviors, (iii) characterization of LC responsiveness to target cues during a vigilance task in relation to tonic LC activity and behavioral task performance, and (iv) specification of short- and long-term relationships between tonic LC activity and stably focused vs. labile attention.

1. Improvement of techniques to record LC discharge in behaving monkeys. Recording from small, deeply located structures in the brain poses significant technical problems. As the reliability of the technique and quality of data obtained are of the utmost importance in recordings from primate, we devoted considerable time and effort during the last funding period to improving our techniques. Novel and original methods for recording from the LC (including a newly designed microdrive, infrared video eye movement monitoring, alignment frame allowing X-rays in stereotaxic planes, and data acquisition and analysis procedures) were implemented,

tested and further adjusted during all successive recordings. This new technology markedly improved the quality of recordings and heightened the yield of LC neurons recorded. Some of these technical advances are described below

We have developed techniques for recording stable unit activity from primate LC using microwire electrodes (25 µm diameter). In our studies, such electrodes were additionally improved by grinding the tip of the wire to a conical shape. These electrodes yield stable recordings of high signal/noise (better than 3/1) from single neurons in LC for several hours in the waking monkey performing a vigilance task. We find this technique to be much better than conventional etched tungsten microelectrodes for recording LC neurons.

We have also designed and constructed a novel microwire holder and advancer for recording unit activity, and developed improved methods of localizing LC. This recording device is especially suited for penetrations into deep brain structures in behaving animals. In brief, a screw-driven microdrive assembly is attached to a small stereotaxically implanted cylindrical pedestal, with the guide cannula stereotaxically positioned 5 - 8 mm above the LC. This design allows small but accurate repositioning of the guide cannula between recording sessions, permitting rostrocaudal and mediolateral adjustments in the initial stereotaxic position so as to precisely localize LC, and allowing multiple penetrations throughout different areas of the LC nucleus to record from a large number of cells in each hemisphere. Due to the abundance of easily identifiable electrophysiological landmarks in the vicinity of LC (auditory responses in inferior colliculus; cell activity with eye movements in the trochlear and abducens nuclei; cell activity with jaw movements in the Mesencephalic V nucleus; large, fast spikes with distinctive complex spike bursts in the cerebellum), and the readily recognizable discharge characteristics of LC neurons, it typically takes only a few penetrations to locate LC after the surgery. LC neurons are readily identified by their distinctive broad spike waveforms, slow steady discharge, marked decrease in activity with drowsiness, and biphasic (excitatory-inhibitory) responses to novel stimuli. As changing electrode tracks does not entail exposing the dura or brain tissue, the possibility of infection is greatly reduced. In our studies using these techniques, unit recordings have been obtained from individual animals for over 6 months with no signs of infection. Also, as only 1 or 2 microwires (25 µm dia.) pass through the region of LC, damage to this area is slight even with multiple penetrations; no gross damage has been observed in histology from the LC in our previous experiments. The device also allows for replacing damaged electrodes, or switching between single- or multiple-wire electrodes, stimulating electrodes or "chemtrodes" (combined recording/infusion electrodes). *Data have been obtained from more than 200 LC neurons in behaving monkeys with these new techniques.*

We have designed and implemented an alignment frame that permits X-ray images to be made in stereotaxic planes. This device is a simple Plexiglas frame that fits onto the A-P bars of a Kopf stereotaxic frame, over the animal's head. The fixation post is positioned in a post-holder, which is then positioned in the X-ray frame so that the post is properly positioned on the animal's head. The post is then cemented to the animal's head, and the post-holder is cemented to an opening in the top of the frame. After the cement hardens, the post is released from the post-holder, and the X-ray frame is removed. The post-holder remains cemented to the X-ray frame throughout experiments for the monkey; each monkey has his own X-ray frame (they are recycled after sacrifice). At any later time, the animal can be re-anesthetized with ketamine and placed back into the X-ray frame by inserting the head-mounted post into the post-holder. This places the animal's head in stereotaxic position with respect to the X-ray frame. In making X-ray photographs, the alignment pins and ear bar markers (inserts temporarily placed in the animal's auditory meatus) are used to align the frame with respect to the camera. The ear bar inserts then are used to measure stereotaxic positions in the X-rays. We have found this technique, combined with the distinctive characteristics of LC neurons and the landmarks around LC, to be very useful in guiding our initial adjustments to the LC cannula, and to shortening the time that is required to find LC with recording electrodes after surgery.

2. Monkey LC discharge during naturalistic behaviors, and in response to unconditioned sensory stimuli. As previously reported in various species, monkey LC neurons decreased activity with decreased arousal (drowsiness) and during certain other behaviors characterized by

lowered attentiveness to the sensory environment (e.g., grooming and eating). Abrupt transitions in behavioral state to increased arousal were consistently associated with (preceded by) increased discharge of LC neurons. As found for other species, LC activity in monkey was most phasically active during such state transitions to high vigilance, and was highly correlated with orienting behaviors.

LC activity in monkeys was also activated by novel or intense unconditioned sensory stimuli. Similar to results in rat and cat, we found that monkey LC cells responded to such stimuli with a brief excitation following by a more prolonged period of diminished activity. As with spontaneous discharge, sensory-evoked LC activity was most intense for stimuli associated with an orienting behavior indicating increased vigilance; conversely, periods of low vigilance were associated with reduced sensory responsiveness.

These observations for sensory LC responses in monkey are consistent with the overall observation that LC neurons were most active, and responded most intensely to stimuli, in association with orienting behaviors and an apparent increase in vigilance. As conspicuous or complex stimuli most consistently elicited these behavioral responses and associated state changes, the above data for stimuli effective in driving monkey LC fit with our previous behavioral analyses of rat LC activity. Overall, these data indicate that sensory stimuli effective in eliciting LC discharge have specific attributes. It appears that LC is geared to respond to stimuli that are conspicuous to animal: stimuli which by their physical or behavioral properties evoke a change in attention.

3. Discharge of monkey LC neurons during a sustained attention task. If the LC regulates vigilance and attention to salient cues as we have proposed, we reasoned that it should respond not only to physically intense stimuli but also to conditioned, meaningful stimuli that require an immediate behavioral response but that are not conspicuous by virtue of their physical attributes. We tested this prediction in recordings of LC neurons in waking cynomolgus monkeys performing an oddball visual discrimination task. In this task, the animal was required to continuously depress a pedal and attend to visual cues. In most of our recordings, the animal was also required to foveate a small spot in the center of the video display to initiate each trial, thereby ensuring attentiveness to the task. Release of the bar within 500 msec after a target cue (vertical or horizontal bar) was rewarded by juice; incorrect releases or misses were followed by a time out. Target cues were presented randomly on 10% or 20% of trials, and the non-target cue was presented on 90% or 80% of the trials. Thus, this task required the monkey to attend over a long period of time (a single session often lasted one hr), withhold responses to the frequent nontarget cues, and respond selectively to infrequent target stimuli. This task is similar to those used in human studies of vigilance and sustained attention.

Single- and multi-cellular activity were recorded from 161 LC neurons in 4 Cynomolgus monkeys performing this vigilance task. We also recorded cortical event-related potentials (ERPs) in response to the sensory cues, as previous work in humans and in monkeys indicated that such slow-wave activity may signal attentional processing in the brain. Initial results of these studies are found in our recent publications. Impulse activity of LC neurons was stable during performance of this behavioral task; single cells could be routinely tracked for hours. Peri-stimulus time histograms (PSTHs) were generated for target stimuli which were followed by a lever response within the allowable delay (hits), for nontarget stimuli followed by a lever release (false alarms), for target stimuli that did not elicit lever responses (misses), for lever responses regardless of stimuli, and for delivery of juice.

Analyses of these histograms across cells revealed a great deal of specificity in activity of LC neurons during this task. Only one cell had activity specifically related to lever release, and only one cell exhibited no response to any parameter examined. The largest category of cells (81/134) were activated selectively by target stimuli but not by nontarget stimuli, lever release or juice delivery; 9/134 cells were selectively inhibited under the same set of circumstances. Twenty-six of the 134 cells were activated by both target and nontarget stimuli, but for these cells responses to target stimuli were substantially more robust than nontarget-elicited activity . These results indicate that LC neurons are responsive to non-conspicuous stimuli that are meaningful by virtue of conditioning, and that require an immediate response.

Recordings during reversal training further supported these conclusions. Within 15 min of reversing target and non-target cues cells terminated their response to the previous target stimulus and began selectively responding to the new target (previously nontarget) cue. Thus, these responses were specifically related to the meaningfulness of the stimuli, not to their physical attributes. Interestingly, these changes varied closely with behavioral performance, so that responses to the new target cue increased (and responses to the new non-target cue decreased) as the percentage of correct behavioral responses to the new target cue increased (and behavioral responses to the new non-target cue decreased). Together, these results are consistent with the possibility that phasic, sensory responses of primate LC neurons has a role in facilitating responses to significant sensory stimuli.

In addition, cortical ERPs exhibited a similar set of properties. That is, long-latency ERPs (200-300 msec) were selectively elicited by target, but not by non-target, cues. These potentials also reversed with behavior and LC responses during reversal training, so that the long-latency ERPs came to be selectively elicited by the new target cue. These results are consistent with the possibility that LC responses to target stimuli may participate in the generation of the long-latency ERP activity. This would be consistent with other results indicating that LC lesions decrease the amplitude of ERPs in monkeys.

Therefore, there is a close relationship among LC-NE neurons, cortical ERP activity, and behavioral responding to meaningful sensory cues. These results indicate that LC responses can be conditioned to salient stimuli and events in the environment, a potentially important attribute for understanding the role of this system in attentional processing.

4. Fluctuations in monkey LC tonic and sensory evoked activities are associated with alterations in focused attentiveness and task performance. LC tonic activity and task performance - In our recent studies we have observed that all 10 LC neurons analyzed from selected long-duration recordings alternated between 2 discrete levels (rates) of spontaneous activity, with each level lasting tens of min. These levels changed in an abrupt "step"-like fashion). In some of our recordings lasting for several hours, LC neurons switched between 2 levels of long-term discharge several times. The difference in discharge rates of these levels was small, in the range of 1 - 2 spikes/sec, but nevertheless quite conspicuous. As described in a preliminary report, these different levels of LC discharge were closely associated with differences in behavioral performance on the vigilance task described above. The periods of elevated LC activity were consistently accompanied by decreased vigilance performance, caused primarily by an increase in the rate of false alarms (lever responses to nontarget stimuli) and increased latencies of bar release following target stimuli, another indication of decreased vigilance performance. In addition, a vigilance decrement is evident for epochs of both low and elevated LC discharge; the major change in vigilance behavior during elevated LC activity is lower *overall* performance than during epochs of lower LC activity. Thus, during the high resting level of LC basal discharge animals responded indiscriminately by eliciting more responses for non-target stimuli, and also exhibited longer latencies in response to target stimuli, perhaps reflecting lower overall vigilance performance. There is also a suggestion in of a more rapid vigilance decrement during epochs of higher LC activity. Additional cases are needed to confirm this possibility. The rate of correct responses ("hits") did not increase with the increased false alarm rate during periods of higher LC activity, as might be expected. In fact, hit rates decreased slightly at these times. Analyses using signal detection theory indicate that during periods of higher LC activity the discriminability of stimuli (d' factor) decreased, while the animal's criterion for responding (ß factor) remained roughly the same as for intermediate levels of activity. One interpretation of these results is that during the higher LC activity the animal was less attentive to the task stimuli (making it more difficult to discriminate target from non-target stimuli), but that his tendency to respond (response criterion) did not change from that of intermediate LC rates. If this analysis is borne out in additional cells and animals, it may mean that LC activity is more involved in the input (sensory) aspects of attention than in the output (motor response) components.

LC fluctuations and changes in focused attentiveness - We also analyzed the frequency with which the animal successfully visually fixated the fix spot, required to initiate each trial. As in

other experiments using this method, foveation of the fix spot in this task is effortful and is a measure of the animal's attentiveness and engagement in the task. As described in a preliminary report, epochs of increased LC activity corresponded to decreased frequencies of fixation; conversely, successful fixation increased during epochs of lower LC discharge. This inverse relationship between LC activity and visual fixation was found to be highly statistically significant for every cell tested using correlation analysis. Note, however, that very low LC discharge was consistently associated with drowsiness as previously reported (described above), and corresponded to low (or no) foveation and little overall task performance. Thus, tonic LC activity in these experiments was related to attentiveness to the task by a curvilinear (inverted U) relationship, with best task performance corresponding to an intermediate level of tonic LC discharge. These results were not expected, and imply that *focused attention corresponds to an intermediate level of LC activity*.

It is possible that these changes in LC activity cause the changes in attention. However, it is also possible that the altered LC activity is not causative of, but rather results from, the changes in attention. Experiments are underway to discern these functional relationships by locally activating or inactivating LC neurons during attentional tasks and measuring the effect on task performance. Our working hypothesis is that very low activity (during drowsiness) provides too little vigilance or alertness for task performance, while high activity results in a level or type of vigilance that is not conducive to focused attention (required for task performance). Specifically, we propose that elevated LC activity may promote a mode of scanning or labile attentiveness, in which the attention span is short and easily altered by exogenous stimuli. This relationship resembles previous arousal models of vigilance function, and may offer a neural substrate for the curvilinear Yerkes-Dodson relationship between arousal and performance.

In addition to the long-term changes in resting discharge described above, LC neurons also exhibited short-lasting fluctuations in activity (epochs 30-60 sec long). These transient discharge levels were too brief to allow ready analysis of different false alarm rates or bar lease latencies as found for the long-term discharge levels described above. However, we found that these short-term changes in LC tonic discharge were often correlated with differences in short-term attentiveness during the task as reflected in different frequencies of visual fixation, similar to the relationship seen with longer-term changes in LC activity described above. Thus, even brief elevations in LC discharge were typically associated with decreased fixation of the fix spot. We have also examined LC activity as a function of simple eye position or movement, and have found no consistent relationship. We have further analyzed short-term changes in LC activity and foveation frequency to ascertain whether changes in LC activity anticipate, and may therefore cause, changes in attentiveness as reflected in successful foveation. To this end, we have analyzed LC spike patterns for the occurrences of bursts. Our preliminary results indicate that a brief pause in LC activity is associated with an increase in foveation frequency, while a burst of LC activity is followed within a few hundred msec by decreased foveation frequency. These results indicate that altered LC activity precedes the associated change in foveation, and are consistent with (but do not prove) the possibility that the LC may cause the change in attentiveness. As noted above, experiments proposed in Aim 1 will directly address this issue further using direct manipulations of LC activity.

Fluctuations in tonic LC activity and changes in LC sensory responsiveness - Analysis of LC responses to target stimuli during the longer epochs of different tonic activity revealed another surprising but marked relationship. Periods of elevated resting activity in a typical LC neuron were consistently associated with decreased responsiveness of that neuron to target stimuli in the vigilance task; the phasic activation of LC neurons typically seen for target stimuli (described above) was observed predominantly during epochs of intermediate tonic LC discharge and best behavioral performance in all 10 cells examined to date. Thus, elevated basal LC discharge corresponds to both decreased behavioral performance (due to indiscriminate responding, long response latencies, and labile or unfocused attention) and decreased phasic activation of LC neurons by target stimuli. Additional analyses are underway to fully evaluate these findings, but they suggest that both phasic evoked responses as well as tonic discharge levels of monkey LC neurons may affect attentional performance. As noted above, manipulations of phasic LC

activity are proposed to determine the causal role of these target responses in vigilance performance.

5. *White noise transiently activates LC neurons and disrupts attentiveness.* In preliminary studies, LC neurons in one animal were recorded while the animal performed the above vigilance task and white noise was briefly presented (100 db, 5-15 min). There was a transient activation of LC neurons during the noise stimulus, but this quickly subsided even though the noise was still present. In parallel with the increase in LC activity, the frequency with which the animal foveated the fix spot decreased, reflecting decreased attentiveness to the task perhaps in response to distraction by the onset of the noise. Additional presentations of the noise on the same day yielded less or no response in LC activity or behavior, suggesting that habituation to the noise in both cellular and behavioral measures was rapid during task performance. The effects of white noise will be examined in additional LC cells in the proposed studies. In addition to examining effects of brief noise presentation, we will also test whether prolonged exposure (>30 min) exerts additional effects on LC activity or behavior not seen in the brief presentations. We will also examine effects of noise on a task that measures attentional lability, the attentional disengagement task.

6. *Acute morphine decreases tonic activity and induces pronounced oscillation of LC discharge in the waking monkey.* LC neurons were recorded from waking, chair-restrained cynomolgus monkeys before, and for 0.5 - 4 h after, i.m. injections of morphine sulfate (0.3 to 10 mg/kg). As shown in the attached publication [22], tonic discharge of each LC neuron tested (n=11) decreased after morphine injection; this effect appeared to be dose-dependent for the range of 0.3-3.0 mg/kg. Unexpectedly, these same doses of morphine also induced a pronounced burst-pause discharge pattern in all LC neurons recorded. The bursts in activity corresponded to (and anticipated) orienting behaviors and increased arousal, whereas pauses were associated with apparent sedation. This was demonstrated using a burst analysis of LC activity, where bursts of LC activity after morphine were closely associated with pupillary dilation. Closer analysis revealed that the burst-pause pattern in LC activity was regular, with a period of about 15-35 sec. This observation was confirmed by autocorrelogram analysis. These results indicate that acute opiates may exert a dual effect on LC neurons in waking animals: inhibition of discharge by direct effects on LC cells, and phasic activation mediated by excitatory afferents to the LC. These short-term changes in LC discharge after morphine resembled in frequency the short-term changes described above during the vigilance task, except that the amplitudes of these changes were much greater after morphine. It is interesting to compare the behaviors associated with these fluctuations in discharge in the two conditions: LC bursts after morphine gave rise to apparently increased vigilance while elevated activity in non-opiate testing was associated with a decrease in focused attention. These may be similar effects, in that in both cases the elevated activity may be associated with more labile, less focused attention. It should be noted, of course, that these behavioral observations are not strictly comparable; for example, animals consistently stopped performing the vigilance task after even low doses of opiates. The relationship between these two fluctuations in LC activity remains to be established.

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34. Shiekhattar, R. and Aston-Jones, G., Sensory responsiveness of brain noradrenergic neurons is modulated by endogenous brain serotonin. Brain Res. (in press)..

35. Akaoka, H. and Aston-Jones, G., Indirect serotonergic agonists attenuate hyperactivity of brain noradrenergic neurons during opiate withdrawal: clinical implications. Neuroscience (in press).

36. Shiekhattar, R. and Aston-Jones, G., Regulation of the spike afterhyperpolarization in locus coeruleus neurons by a non-protein kinase-dependent action of cyclic AMP Neuroscience (in press).

37. Charlety, P.J., Chergui, K., Akaoka, H., Saunier, C.F., Buda, M., Aston-Jones, G. and Chouvet, G., Serotonin differentially modulates responses mediated by specific excitatory amino acid receptors in the rat locus coeruleus in vivo (submitted to Europ. J. Neurosci.).

38. Shiekhattar, R. and Aston-Jones, G., Modulation of opiate responses in brain noradrenergic neurons by basal and stimulated cAMP-dependent protein kinase: changes with chronic morphine (submitted to Neuroscience).

39. Shipley, M.T., Fu, L., Ennis, M. and Aston-Jones, G., Distribution of locus coeruleus extranuclear dendrites: Immunocytochemical LM and EM studies (in preparation for Brain Res.).

40. Aston-Jones, G., Akaoka, H., Shipley, M. and Zhu, Y., Selective induction of Fos protein in subsets of catecholamine neurons during opiate withdrawal (in preparation for J. Neurosci.).

41. Grenhoff, J., Nisell, M., Ferre, S., Aston-Jones, G. and Svensson, T.H., Noradrenergic modulation of midbrain dopamine cell firing elicited by stimulation of the locus coeruleus in the rat (submitted to J. Neural Transmission).

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Drolet, G., Van Bockstaele, E.J., Akaoka, H. and Aston-Jones, G. Enkephalin afferents to the locus coeruleus from the rostral medulla. 3rd IBRO World Congress of Neuroscience Abst. 382 (1991).

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Aston-Jones, G. and Shiekhattar, R. Attenuation of after-hyperpolarization in locus coeruleus neurons by cAMP is independent of protein kinase activation. Soc. Neurosci. Abstr. 18: 103 (1992).

Kubiak, P., Rajkowski, J., Luthin, G. and Aston-Jones, G. Tonic and sensory-evoked activities of noradrenergic locus coeruleus (LC) neurons in primate vary with discrimination performance in a vigilance task. Soc. Neurosci. Abstr. 18: 538 (1992).

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Robine, V., Valentino, R., Aston-Jones, G. and Lehmann, J. Norepinephrine release elicited *in vivo* by local NMDA receptor stimulation. Soc. Neurosci. Abstr. 18: 915 (1992).

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Shiekhattar, R. and Aston-Jones, G. Enhancement of opiate responses in brain noradrenergic neurons by cAMP-dependent protein kinase: Changes with chronic morphine. Intl. Catecholamine Symposium Abstr. 7, 1992.

Aston-Jones, G., Rajkowski, J., Kubiak, P., Alexinsky, T., Shipley, M. T., Ennis, M., Akaoka, H. and Astier, B. From the medulla to attention through the locus coeruleus: Cellular physiologic and anatomic studies. Intl. Catecholamine Symposium Abstr. 7, 1992.

PROFESSIONAL PERSONNEL:

Gary Aston-Jones, Ph.D., Professor (Principal Investigator)	20%, 3 years
Tatiana Alexinsky, Ph.D. Research Associate	100%, 0.5 year
Janusz Rajkowski, Ph.D., Research Assistant Professor	100%, 3 years
Piotr Kubiak, Ph.D., Postdoctoral Fellow	100%, 2 years

INTERACTIONS:

Invited presentations at national and international meetings:

European Winter Conference on Brain Research, Les Arcs, France, March, 1990.

International Symposium on the Neurobiology of the Locus Coeruleus, Post Falls, Idaho, May, 1990.

XIIIth Congress of the International Primatological Society, Kyoto, Japan, July, 1990.

European Brain and Behavior Society Symposium, "Functions of the forebrain cholinergic and noradrenergic systems", Stockholm, Sweden, September, 1990.

McDonnell Foundation Workshop on Emotion, Montauk, New York, September, 1990.

Chairman and speaker, "The Ventrolateral Medulla: A Site for Integration of Pain, Sympathetic Activity and Arousal", Special Panel, Winter Conference on Brain Research, Vail, Colorado, January, 1991.

Chairman and speaker, "The Ventrolateral Medulla: A Site for Integration of Pain, Sympathetic Activity, Respiration and Arousal", Workshop, IBRO 3rd World Congress of Neuroscience, Montreal, Canada, August, 1991.

Chairman and speaker, "The Locus Coeruleus-Norepinephrine System: New Basic and Clinical Perspectives", Panel, American College of Neuropsychopharmacology (ACNP), San Juan, Puerto Rico, December, 1991.

Symposia presentations (2), Seventh International Catecholamine Symposium, Amsterdam, June, 1992.

International Research Conference of the American Academy of Osteopathy, on *Nociception and the Neuroendocrine Immune Connection*, Cincinnati, Ohio, June, 1992.

American College of Neuropsychopharmacology (ACNP) Panel, "Neural Mechanisms of Learning and Memory: Relevance to the Consequences of Severe Psychological Trauma", San Juan, Puerto Rico, December, 1992.

Chairman and speaker, "Catecholamines and Attention: New Basic, Clinical and Modeling Approaches", Workshop, Winter Conference on Brain Research, Whistler, British Columbia, January, 1993.

The above does not include more than 15 invited seminars at other universities.